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Transfer factor in malignancy

By Giancarlo Pizza[1], Caterina De Vinci[1] and H. Hugh Fudenberg[2]

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1 Introduction

Cell-mediated immunity (CMI) plays an important role in controlling the proliferation of tumor cells. Since transfer factor (TF) is reportedly able to increase CMI, it was tempting to plan clinical trials whereby transfer factor could be used to increase cancer patients' cellular immune response to their tumor cells.

The first such clinical study was published by Thompson in 1971 [1]. Numerous reports have followed since, but most studies, like the original one, are criticizable on several accounts: they were uncontrolled, the number of subjects involved was small, more often than not the patients were in a stage of advanced disease and their tumor load was important, the follow-up period was short. Furthermore, it is impossible to draw any, even preliminary, conclusion when results of different centers pertaining to the same tumor type are contradictory. The lack of a product whose activity is known and can be standardized makes it difficult to decide whether the reported failures are due to the lack of activity of the transfer factor used, to the protocol of its administration, or to the ineffectiveness of this kind of immunotherapy for the type of tumor treated.

Nonetheless, and despite these criticisms, several studies produced encouraging and sometimes unequivocal results which prompted further clinical trials for other types of cancer [2–6]. Furthermore, some laboratory studies were showing that incubation of unreactive lymphocytes with TF was able to induce recognition of TAA.

In 1976, Byers et al. [7] were among the first to produce evidence for such in vitro transfer of reactivity to tumor cells. They extracted transfer factor from osteosarcoma patients whose lymphocytes were showing cytotoxicity against osteosarcoma cells. The dialysate was then used to inject osteosarcoma patients whose lymphocytes became subsequently cytotoxic and able to kill autologous tumor cells.

At that time, we have also shown that specific transfer factor, obtained from patients with high levels of CMI to tumor-associated antigens (TAA) of bladder carcinoma – as assessed by the leucocyte migration inhibition test (LMT) – was able to transfer to the leucocytes of the recipient, by in vitro incubation or by in vivo injection, the reactivity observed in the TF donor [8–10].

Such observations encouraged tumor immunologists to treat cancer patients with transfer factor in the hope that the modulation of their immune response against TAA could counter the tumor growth.

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However, the distinction between specific and non-specific transfer factor was not always made at the time and clinicians would use the so-called non-specific transfer factor obtained from pools of buffy coats from blood donors. But even when it was accepted that specific transfer factor was more desirable than non-specific, its scarcity-obliged investigators to use dialysates from pools of leucocytes.

Some investigators would nonetheless argue that the clinical benefit of transfer factor may be related to the non-antigen-specific immunopotentiating effects of the leucocyte dialysate, rather than to the specific transfer of cellular immunity. Furthermore, one cannot exclude that the so-called non-specific transfer factor may, by chance, contain information for tumor specificities relevant to the tumor type of the recipient.

Indeed, using such "non-specific" transfer factor, Krown and coworkers reported in 1978 two tumor regressions in 18 patients with advanced cancer and they also observed that the treatment was associated with at least a temporary increase of delayed hypersensitivity reactions in 12/17 patients tested. They concluded that lymphocyte dialysates may augment delayed hypersensitivity in patients with advanced cancer, and that some of the observed effects may be non-specific, i.e. unrelated to transfer of information pertaining to TAA [11].

Results of clinical trials using transfer factor therapy in various malignancies have been extremely variable. In non-randomized trials, more than 300 patients have been enrolled, and clinical benefit has been reported in about 1/3 of them. Results of randomized studies also vary: in some, beneficial clinical effects, measured by increased disease-free interval and prolonged survival, have been claimed, whereas in other studies, transfer factor has been reported to be of no clinical benefit. Finally, some reports suggest that certain patients receiving transfer factor do not do as well as those receiving placebo, although this type of observation has never reached statistical significance.

Since there are many variables in the design of transfer factor clinical trials, it is impossible from their analysis to identify the key factors responsible for the observed success or failure. For instance, the state of disease, previous and/or concomitant chemotherapy and/or radiotherapy vary widely and their impact in the clinical and immunological response is unclear. Obviously, the source, but also more

often than not the dose of transfer factor used vary from one clinical study to another, and this is another serious handicap for establishing valid comparisons and overall evaluations of efficacy.

Finally, it should be reminded here that the methods of preparation of transfer factor also vary and the products used in the different studies are not a priori comparable nor are there reliable tests allowing measurements of specific activity and thus making comparisons between laboratories possible.

Several tumor types have been treated with transfer factor, but because of the uncertainties mentioned above, it is not established which tumors only partially respond to this therapy and which are totally unresponsive.

The results obtained in the most important clinical studies in the last twenty years are briefly described hereafter.

2 Lung cancer

One of the most important studies on lung cancer was published by Whyte et al. who, between 1976 and 1982, treated sixty-three patients with bronchogenic carcinoma with TF obtained from apparently healthy blood donors. These patients had previously undergone pulmonary resection, mediastinal lymph node dissection, and, when it was necessary because of mediastinal lymph node involvement, mediastinal irradiation. Patients were randomized into two groups. A group of 28 patients received pooled TF 3 months after surgery, whereas 35 patients of the control group received saline injections. Follow-up was completed in 1990. In the transfer factor group, the 2-, 5-, and 10-year survival rates were 82%, 64%, and 43% respectively, whereas in the control group they were 63%, 43%, and 23%. Survival in patients receiving TF was consistently better than in the placebo group for both adenocarcinoma and squamous cell carcinoma.

Although these long-term results were not statistically significant, using survival analysis ($p = 0.08$), the authors were able to confirm their previous observations in a smaller number of lung cancer patients treated for a short-time period suggesting that administration of TF can enhance CMI and improve survival in patients with bronchogenic carcinoma [12].

In a different study, Busutti et al. also reported a beneficial effect of TF administration in a series of 26 non small cell lung carcinoma patients (NSCLC) [13].

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In order to evaluate the efficacy of adjuvant TF immunotherapy after surgery, Fujisawa and coworkers treated, from March 1978 to October 1984, 263 primary NSCLC cancer patients who had undergone pulmonary resection. They showed that in patients in stage I of the disease (but not in stages II, III and IV), the effect of TF was statistically significant, thus suggesting that TF can only suppress "micrometastases" existing at the time of surgery. The authors also immunized in vitro lymphocytes from household contacts (i.e. family members) with IL-2 and mitomycin-treated lung cancer cells. These T-lymphocytes showed considerable cytotoxic activity against the target cells, which were used for in vitro sensitization, and their dialysate showed capability to transfer specific cytotoxic activity against lung cancer cells [14–16].
In another controlled trial on 102 lung cancer patients, randomized after surgery, the TF group of 44 patients was compared to a control group of 47. Again, the survival of the TF group was significantly better than that of the control in patients of stage I ($p<0.05$). However, there was no significant difference in patients of stage II, III or IV. Furthermore, significant differences were found between the TF and control groups in patients who had undergone curative resection ($p<0.05$). The authors concluded that TF seems to inhibit postoperative recurrence and appears to be an effective post-operative adjuvant immunotherapeutic tool for primary resected adenocarcinoma of the lung patients, especially at the early stage [16–23].
In contrast to the work cited above, Kirsh and coworkers observed good clinical results also in patients of advanced stages. In 28 patients with lung cancer (treated with 1 ml of TF extracted from the blood of normal individuals of 3-month intervals), they showed a significant increase of survival, when the TF-treated group was compared with 35 randomized control patients. The 2-year survival was 78% for the TF-treated patients and 46% for the control group ($p<0.04$) [24–25].

3 Osteosarcoma

The work of Fudenberg's team produced solid evidence concerning the ability of TF from selected donors to increase the CMI responses in human osteogenic sarcoma patients [7, 26–28]. An animal Osteosarcoma Tumor model using TF has also been reported by the same team [29]. In addition, in another section of the present volume, very

important results are also reported by Fudenberg, regarding the prevention of human osteosarcoma lung metastases in hamsters pre-treated with specific TF.

Levin et al. administered specific TF after surgical removal of the primary tumor to patients without apparent metastases. Five out of 6 patients were still alive 5 years later, after 2 years of TF administration. This survival was significantly better than no treatment or other treatment, when compared to historical controls ($p<0.008$) and it was attributed to the prevention of lung metastases [30]. Their results were confirmed by others [31–33]. Similar results were obtained in an animal model [34].

The first report of a randomized postsurgical clinical trial with TF versus combination chemotherapy in osteogenic sarcoma was published by Ivins and coworkers in 1976 [35]. Twenty-six patients with osteosarcoma were randomized to receive either TF or combination chemotherapy. Eight of 14 patients who received transfer factor converted their skin test markers demonstrating biological activity of the TF. Of these eight patients, all were alive and four free of disease, in a 6-month follow-up. Of the 18 patients who received combination chemotherapy, 14 were alive and 12 free of disease, during the same observation period. Laboratory tests subsequently showed that transfer factor appeared to enhance CMI, although no predictive correlation could be established between laboratory results for each patient and his clinical response to TF treatment. However, the limited number of patients and the short duration of the study did not allow to draw definitive conclusions [35].

Gilchrist and coworkers [36] proposed an adjuvant TF therapy for the treatment of non-metastatic osteogenic sarcoma after apparent complete surgical ablation of the primary tumor compared to combination chemotherapy. From a total of 32 patients assessed, 22 received chemotherapy; in this group three died of drug-related complications and six were alive without disease recurrence between 260 and 673 days after operation. The ten patients in the transfer factor group all converted their skin tests, and five were alive without recurrence 420–753 days after operation. No significant difference was seen between the two groups with respect to disease-free survival [36].

4 Melanoma

Several clinical trials have been carried out in patients with malignant melanoma. The results have been inconclusive and sometimes contradictory. Thus, in one of these studies, performed in double blind on 68 patients of stage I and II as adjuvant therapy after surgery and having as end-point the time of stage III metastases, a trend in favor of the placebo group was observed [37].
In contrast, in a non-randomized clinical trial with a control group of 46 patients, the results were more encouraging. One hundred patients at stage I melanoma were treated with transfer factor and monitored with a median follow-up period of 30 months. The survival rate was 99% at five years compared to 69% in the control group. These results suggest that TF immunotherapy may be a valuable adjunct in the treatment of patients with high risk stage I melanoma [38].
Gonzalez and Spitler treated with surgery and TF nine patients with resectable pulmonary metastases of malignant melanoma. Twelve months after thoracotomy, they were all alive and after a median follow-up of 20 months, only one patient had died. Historic controls of patients from other centers treated with surgery alone showed a significantly lower survival rate ($p < 0.025$). Recurrence rates tended to be lower in the TF group, but no significant difference was found with historic controls. The data seem to suggest that transfer factor may prolong survival in patients with a small residual tumor burden and furthermore, this type of immunotherapy produces better results in lung metastases [39]. In a randomized controlled trial of stage II malignant melanoma, Bukowski et al. were unable to find any statistical difference in survival between the 18 patients treated with TF compared to the 18 patients of the control group, despite the fact that the median disease-free intervals were respectively 12.0 and 10.0 months, and survival 40.8 and 27.0 months. Nine TF-treated patients and four control patients remained alive 68 months after the end of the treatment [40].
However, no improvement was seen when TF was added to the protocol of chemotherapy and BCG immunotherapy used for the treatment of disseminated malignant melanoma [41].
In conclusion, although some encouraging results have been observed in certain clinical studies when TF was used for the treatment of melanoma patients, further clinical trials should be planned using

selected specific TF, covering several melanoma antigenic specificities before any meaningful conclusion can be drawn.

5 Papillomatosis of larynx

Although some anecdotal encouraging responses were observed in some patients [42–45], no controlled clinical trials have been carried out on papillomatosis of the larynx. Borkowsky reported complete remission of pulmonary metastatic lesions in a 6-years-old girl after 2 years of treatment with TF prepared from her mother. A computer tomographic scan performed after four months of therapy revealed almost complete resolution of her pulmonary lesions [42]. Two other cases have been reported by Ortiz and coworkers [43].

6 Nasopharyngeal cancer (NPC)

Numerous studies substantiate the hypothesis of the role EBV in the pathogenesis of BL and NPC, thus making the idea of potentiating the CMI against the EBV antigens in these patients in order to prevent relapses a plausible one [46–48].
However, the difficulties in the choice of the best TF donor should be underlined. In view of the preparation of an active anti-EBV TF for in vitro replication by the LDV/7 lymphoblastoid cell line, we used different sources: a) nude mice immunized with EBV, b) a patient who had recovered from infectious mononucleosis and c) a NPC patient in remission showing a very strong CMI, as assessed by lymphocyte stimulation and leucocytes migration inhibition in presence of EBV-superinfected Raji cells. Transfer factor extracted from PBL of the NPC patient was the most active in transferring both in vitro and in vivo reactivity to EBV antigens. These data suggest that negative results obtained in clinical trials of NPC using TF from other sources might be artifactual because of the lack of TF reactivity [46]. The clinical results obtained with an active anti-EBV TF in Burkitt's Lymphoma [47] makes this assertion so much more credible.
Goldenberg et al. treated 100 NPC patients in a cooperative trial with EBV-specific transfer factor [48]. The prospectively randomized, double-blind clinical trial was planned to evaluate the effect of immunotherapy with transfer factor as an adjunct to radiotherapy on patients with stage III NPC. The TF was obtained from healthy young adult

volunteers with a proven history of infectious mononucleosis and from healthy blood donors with elevated anti-EBV-capsid-antigen antibody titers. TF thus prepared was previously to its clinical use shown (by the leucocyte adherence inhibition test) to convert in vitro NPC patients' leucocytes. When it was administered to NPC patients *in vivo*, it seemed to slow tumor growth, and this was associated with lymphocyte infiltration of the tumor, and recovery of delayed cutaneous hyper-sensitivity reactions [49]. From 1974 to 1977, 100 patients with NPC were entered in the study; one-half of the patients were treated with radiotherapy alone, whereas the other half received radiotherapy and an 18-month course of TF immunotherapy. The patients were followed for at least 5 years. No significant difference in survival or disease-free interval was noted between the two groups. One may conclude that this particular TF preparation and/or the schedule of administration was devoid of any anti-tumor activity [48].

7 Burkitt's lymphoma (BL)

Because in endemic areas BL seems to be reinduced by the persistence of the Epstein-Barr virus (EBV) infection, we thought that the reinforcement of the CMI against EBV in BL patients in remission could prevent late relapses, early relapses being considered as relapses of the primary tumor not eradicated by chemotherapy. Prior to the clinical study we had shown that a specific TF replicated in vitro was able to transfer in rhesus monkeys, both in vitro and in vivo, cell-mediated immune reactivity against membrane antigens induced on Raji cells superinfected with the EBV marmoset strain [46, 50].

Twenty-seven children with abdominal Burkitt's lymphoma (stage III), who had achieved complete remission by standard chemotherapy, entered into a prospective controlled randomized trial of adjunct treatment with EBV-specific TF [46]. Two out of 12 TF-treated patients and 5 out of 11 controls suffered relapses. Time to first late relapse was longer among TF-treated patients ($p = 0.08$), and no late relapses occurred while patients were receiving TF treatment. It thus seems that EBV-specific TF might be useful in the management of endemic Burkitt's lymphoma and also in the treatment of other virus-associated cancers [47].

8 Cervical cancer and household contacts TF

In a prospective randomized double-blind study on 60 patients with invasive cervical cancer, Wagner and coworkers treated 32 women with TF derived from the leucocytes of their husbands, and 28 with placebo. Within the first 2 years after radical hysterectomy, 11 out of 28 placebo and five out of 32 TF-treated patients developed recurrence of malignancy. These data are statistically significant ($p < 0.05$) [51–53]. Vetto et al. also treated 35 patients with various tumor types (viz. melanoma, osteogenic sarcoma, rhabdomyosarcoma, Wilms tumor, renal cell carcinoma, epidermoid carcinoma, colon adenocarcinoma, lymphosarcoma) using TF obtained from donors selected among family members living in cohabitation. The objective of this study was to stimulate CMI to specific tumor antigens by specific TF. Cancer patients not suitable for further conventional therapy were selected for this protocol and TF was administered at 2-week intervals. The TF immunotherapy produced tumor regression in 13 patients, and arrest of metastatic disease and pain relief in 14 patients. Conversion of dermal reactivity to specific TAA was observed during periods of clinical improvement. Despite continued immunotherapy, the duration of clinical improvement was short (2 weeks to 12 months). Seven of the 11 patients not responding to therapy exhibited serum blocking of lymphocyte responsiveness. The results suggest that TF from household contacts can effectively stimulate specific CMI in cancer patients and produce in some cases an inhibition of tumor growth [54].

9 Hodgkin's disease (HD)

Several investigators claimed increase of CMI in patients with Hodgkin's lymphoma. It was assessed by the increase of patients' dermal reactivity to various antigens and/or increase of circulating total T and CD4+ lymphocytes. In contrast the number of NK cells in the peripheral blood does not appear markedly affected [55–58].
Furthermore, a beneficial effect on the impaired functions of mononuclear leucocytes was observed, whereas the decreased phagocytosis and chemotaxis of monocytes was increased almost to the values of healthy controls [56]. The number of T cells bearing histamine and IgG Fc receptors was reduced initially and increased during TF therapy, but this effect was only temporary. Furthermore, non-specific

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TF injections in children with Hodgkin's disease seem to produce an increase in B-lymphocytes, both in percentage and absolute number [59].

Only one randomized clinical trial has been reported by Hancock and coworkers [60]. They prepared TF from 493 buffy coats obtained from healthy donors including individuals convalescent from miscellaneous viral infections. Twenty-two Hodgkin's patients were randomized and subcutaneously injected every 3 months with 1 ml of TF (3×10^8 mononuclear cell equivalent); 25 patients, injected with 1 ml of saline, served as controls. The authors confirmed the increase of skin reactions in anergic patients, but did not show augmentation of other immune parameters neither noticed reduction of the incidence of viral infections [60].

10 Renal cancer

In 1978, in a prospective but not randomized trial on 27 metastatic renal cell cancer patients (MRCC), Bukowski et al. reported some benefit when TF was used for treatment alone or in combination with BCG or chemotherapy [61]. Previously they had treated with TF 5 MRCC patients and observed temporary stabilization of the disease [62]. Two additional patients, without clinically evident metastases, but at a high risk for recurrent disease, were also treated and remained disease-free.

A critical review of immunotherapy of disseminated renal adenocarcinoma was published in 1982 by Montie et al. [63]. Sixty patients with renal adenocarcinoma had been treated in five different immunotherapy protocols consisting of 1) transfer factor, 2) association of TF and BCG, 3) association of TF, BCG, Chloroethylcyclohexy-nitrosurea (CCNU) and megestrol acetate (Megase), 4) association of BCG, CCNU, and Megase, and 5) BCG alone. While this non-specific immunotherapy of renal adenocarcinoma has been associated with documented regression of metastases, response rates were similar to those obtained with hormonal therapy alone. However, because of these results, further clinical studies were undertaken [63].

Thirty-seven MRCC patients, compared to 27 historical controls, were treated with combined immunotherapy including direct lymphatic injection of IL-2 and Lak cells, intramuscular injection of alpha-2a-interferon (10^6 units biweekly) and TF (bimonthly injections of 4×10^8

mononuclear cell equivalent obtained from pooled buffy coats of healthy blood donor). This regimen produced complete and partial remissions of metastases in 34% of the patients treated and stabilized progression of the disease in an additional 8%. The median survival is, respectively, 26 and 27 months for synchronous and metachronous metastatic treated patients against 8 and 14 months for the control group ($p < 0.001$) [64]. While no side effects were noticed in the treated patients, the observed results are comparable to those obtained by intravenous injections of large amounts of IL-2 and Lak cells, a protocol which produced severe adverse side effects [65].

11 Bladder cancer

TF was extracted from lymphocytes of patients with transitional cell carcinoma of bladder (TCCB) and replicated in culture using the LDV/7 lymphoblastoid cell line [66]. The ability of the in vitro replicated TF in transferring sensitivity to TCCB was assessed in LMT using formalin-treated TCCB cells as antigen. It was shown that it was able to transfer not only to leucocytes of healthy blood donors but also to leucocytes of TCCB patients [9].

When TCCB patients were injected with this TF, we observed transfer of reactivity against autologous tumor cells in 6 out of 8 patients and in 11 out of 14 for allogeneic bladder tumor cells [8, 10, 67]. Increase of total number of lymphocytes and T cells was also noticed when their values were low before TF injection, whereas responses to PHA and Con-A appeared also increased after the TF injections [9].

In certain patients evaluation of antibodies against TCCB antigens showed a drop of the antibody titer immediately after the TF injection. This was attributed to the increase of the number of cytotoxic lymphocytes and the subsequent destruction of tumor cells, thus liberating TAA reacting with the circulating antibodies [68]. We must remind, in fact, that 80% of TCCB patients show the bloodstream complement fixing antibodies against their own tumor as assessed by indirect immunofluorescence test on fresh or fixed tumor cells [69]. The same antibodies, harvested by plasmapheresis or produced in vitro, are now used in passive immunoprophylaxis of the tumor [70] and the results on the first 114 TCCB patients appear encouraging. In fact we notice significant reduction of tumor relapse index without noticing any side effect ($p < 0.01$). It is worth mentioning here that we

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Immunotherapy of recurrent, superficial papillary TCCB, using non-specific TF as sole therapy, was also attempted. Only one controlled study was published which fails to show any difference between the TF treated patients and those of the control group [71].

12 Prostate cancer

In a preliminary trial, 7 patients with hormone-resistant metastatic adenocarcinoma (stage D3) were treated monthly with the in vitro replicated TF, used for the treatment of TCCB. In one patient, a complete regression of multiple bone metastases as well as the primary tumor was noticed [72]. These observations were confirmed in a study of 56 same stage prostate cancer patients. The patients received monthly an intramuscular injection of in vitro produced TCCB-specific TF. Follow-up, ranging from 1 to 8 years, showed that complete remission was achieved in 2 patients, partial remission in 6, and there was no progression of metastatic disease in 14. The median survival was 17 months, higher than the survival rates reported elsewhere [73].

Effect of transfer factor on tumor-associated immunity and tumor growth of the Dunning R-3327 G rat prostate adenocarcinoma was reported by Shaw and coworkers [74]. Dunning R-3327 rat prostate adenocarcinoma, and its sublines, represent an experimental tumor model of its human counterpart. In a preliminary study, the effect of TF on tumor-associated immunity and tumor growth and histology of the G subline (a poorly differentiated, fast-growing, androgen sensitive, and poorly metastatic cell-line) was evaluated. TF was prepared from the leucocytes of tumor-bearing animals and non-tumor-bearing animals. Its administration showed no significant effect on the tumor size. The only noticeable effect of TF was the presence of variable and moderate lymphocyte infiltration, necrosis, and degenerative-type cells in tumors of animals receiving TF from immunized animals. The authors conclude that additional evaluation of the effect of TF on other, more immunogenic, cell sublines is needed [74].

13 Epidermodysplasia verruciforme

Vasily and coworkers treated 2 patients with epidermodysplasia verruciformis, a chronic cutaneous infection with a variety of human papilloma viruses using TF obtained from household contacts. One patient with longstanding (30-year) disease and no response to previous therapy, showed gradual and definite resolution of extensive tinea-versicolor-like verrucae planae plaques, as well as tumor lesions scattered over his entire integument. Cessation of TF therapy for a short time resulted in recurrence of partially regressed lesions and also in the development of new tumors. The second patient (grandson of the first) with minimal disease showed no worsening of his condition during TF prophylaxis. Patients showed low numbers of T suppressor lymphocytes, a defect in cell-mediated immunity not previously reported in patients with this disease [75].

14 Childhood leukemia

Few studies were performed on childhood leukemia. De Bruyere and coworkers found an association between long survival in childhood acute lymphoblastic leukemia treated with transfer factor and certain HLA haplotypes. The study was carried out in 116 children. Patients with A2 B12 and/or A2 B40 haplotypes survived longer than patients without these two haplotypes. Since all children were treated with TF obtained from their relatives, it is suggested that children with A2 B12 or A2 B40 haplotypes may respond better to this type of immunotherapy [76]. Unfortunately no other studies to further investigate and confirm this interesting hypothesis have been published.

15 Mycosis fungoides (MF)

In a controlled study, Thestrup and coworkers treated 16 patients with mycosis fungoides with non-specific TF obtained from healthy blood donors as adjuvant therapy to topical nitrogen mustard or PUVA. The clinical evaluation after 2 years of therapy failed to show any effectiveness of the treatment in the control of the disease [77].
In another non-randomized study, TF was associated to oral retinoids in combination with chemotherapy [78]. The patients were divided into two groups, only one received retinoids, whilst both groups

received a 3-drug combination chemotherapy consisting of bleomycin, cyclophosphamide and prednisone. Complete remission, including regression of all signs of lymph-node involvement was observed in 8 of 10 patients of the group treated with retinoids, while none went into complete remission in the control group. All patients in the control group died between 3 and 12 months after the end of therapy, whereas all but one in the group treated with retinoids remained alive. These results suggest that TF in the absence of retinoids has no beneficial effect [78]. Nonetheless, it would be of interest to compare a group of patients receiving TF immunotherapy with a group not receiving this treatment, both groups receiving chemotherapy and retinoids.

Promising results were also reported by Zachariae and coworkers. They treated, using TF as additional therapy to conventional treatment, after the latter had failed, 13 patients with clinically and histologically confirmed MF. Approximately 3 years later, 3 patients were in complete remission, 4 patients were significantly improved and considered as being in partial remission, whereas no change was noticed in 3 patients. The condition of one patient was worse, 1 patient died after discontinuation of the therapy and 1 patient opted out of the study. The number of T lymphocytes, which was low prior to treatment, increased to normal values during TF administration. During the first year a decrease in serum IgE was noticed. The results of the clinical evaluation seem to indicate that TF could be of value as an adjuvant therapeutic agent in MF, although further controlled clinical trials are needed to corroborate this assumption [79].

16 Miscellaneous

Ten patients with breast cancer were treated with TF. Clinical improvement was observed in 2 of 10 patients, whereas increase of skin reactivity was noticed in 3/10 [80–81].

Some encouraging results of transfer factor therapy in Waldenstrom's macroglobulinemia and multiple myeloma were observed by Silverman et al. [82].

Pain relief was observed by Vetto et al. in one patient with epidermoid carcinoma and 75% tumor regression for 3 months in one colon carcinoma patient [83].

Vetto et al. also treated patients with head and neck cancer. The

T-lymphocyte levels increased in 8/38 patients who received non-specific TF, although leucocyte adherence inhibition (a test used to determine tumor immunity) did not occur. No clinical results were reported [83].

17 Conclusions and perspectives

The results of the clinical trials reported here clearly suggest that transfer factor cannot be used as sole treatment of cancer, despite the reported cases of remission. It remains nevertheless that transfer factor can play an important role as an adjuvant to other treatments (i. e. surgery, irradiation, chemotherapy) or in combination with other lymphokines. For instance, we are now using immunotherapy for metastatic renal carcinoma which combines intralymphatic injections of IL-2, [64, 84] α-interferon and specific transfer factor. Our results show that this regimen is at least as efficacious as I.V.IL-2 injections and far less toxic.

Controlled studies are of the essence for future evaluation of the clinical activity of transfer factor. Obviously, a prerequisite for such studies is to dispose of adequate amounts of an active product. Furthermore, for the comparison of studies carried out in different centers using dialysates from various origins, it is essential to adopt the same criteria for measuring in vitro activity. Only one such in vitro test, the leucocyte migration inhibition, has so far been widely used but the results are not always transposable from one laboratory to another.

Furthermore, the choice of the target antigen to determine the activity of the dialysate is important. In our studies with bladder carcinoma, we have been using formalin-treated autologous and/or allogeneic tumor cells, whereas for Burkitt's lymphoma, AIDS and herpes studies, formalin-fixed virus-infected cells have been utilized.

It should be reminded here that for several decades, one of the main curtailments for the clinical use of transfer factor has been the lack of adequate supplies of active material. Indeed, twenty years ago, the only source of transfer factor was human leucocytes. Buffy coats from healthy blood donors were thus used to prepare large amounts of material whose specific activity was unknown. The possibility of encountering the appropriate specificity in a given batch for a given patient was amounted to a lottery draw. At that time (1974), some of us

proposed the use of a lymphoblastoid cell line to replicate transfer factors of known specificity [85]. Multiple reports have now confirmed that such replicated transfer factor carries the original specificity and it is able to transfer it not only in vitro but also in vivo [8–10, 47, 57, 86, 87].

A few years later, the same laboratory showed that animal transfer factor is active in humans [88]. Several studies have subsequently shown that transfer factor from animal origin can be replicated in vitro and that both the in vitro replicated or the animal transfer factor retain their activity even when they are orally administered.

For instance, in an important clinical trial, more than 200 patients suffering from genital, labial or ocular herpes were orally treated using specific anti-HSV transfer factor from bovine origin [89–93, and Pizza et al., unpublished].

Thus, the problem of the preparation of large amounts of active material seemed to be solved. Unfortunately, at that time, transfer factor became compound non grata in the scientific community who is dominated by what some call hard science which denies the right of existence to unexplained phenomena in biology which seem to challenge the day's paradigm and disturb the consensus.

The logical approach to produce sufficient amounts of TF when the antigen is known and readily available, is animal immunization, whereas when the involved antigen(s) is (are) unknown, one can use transfer factor obtained from the patient's lymphocytes and/or those of household contacts. This transfer factor can be subsequently replicated in tissue culture. The in vitro replication allows to replenish the stocks indefinitely when an active transfer factor is found.

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